Epigenetic silencing directs expression heterogeneity of stably integrated multi-transcript unit genetic circuits.

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Public Summary:
We report that epigenetic silencing causes the loss of function of multi-transcript unit constructs that are integrated using CRISPR-Cas9. Using a modular two color reporter system flanked by selection markers, we demonstrate that expression heterogeneity does not correlate with sequence alteration but instead correlates with chromosomal accessibility. We partially reverse this epigenetic silencing via small-molecule inhibitors of methylation and histone deacetylation. We then correlate each heterogeneously-expressing phenotype with its expected epigenetic state by employing ATAC-seq. The stability of each expression phenotype is reinforced by selective pressure, which indicates that ongoing epigenetic remodeling can occur for over one month after integration. Collectively, our data suggests that epigenetic silencing limits the utility of multi-transcript unit constructs that are integrated via double-strand repair pathways. Our research implies that mammalian synthetic biologists should consider localized epigenetic outcomes when designing complex genetic circuit

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